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TITLE: Combination of Extracorporeal Life Support and Mesenchymal Stem Cell Therapy for Treatment of ARDS in Combat Casualties and Evacuation of Service Members with ARDS

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14. ABSTRACT

Transfer of injured service members from the Level 3 combat support hospital to level 4 and 5 medical facilities increase their chance of survival from devastating injuries. Aeromedical evacuation of patients with Acute Respiratory Distress Syndrome (ARDS) is sometimes beyond the possibilities because of limitations providing ventilator support in flight with a possible further deterioration in patient status. Cell based therapy with adult bone marrow-derived mesenchymal stromal cells (MSC) in experimental models of ARDS data suggest that administered allogeneic B-MSCs can mitigate hypoxemia and promote recovery. However, it is unknown how this new form of therapy can be used adjunct to current supportive measures for lung failure. Our objective is to complete a series of preclinical studies in large animal models using extracorporeal membrane oxygenation (ECMO) alone or in combination with MSC in sheep and pigs with ARDS. Our group had completed the first 19 experiments in which we demonstrated that 3.5 ug/kg of LPS infused i.v. to a sheep induces lung injury equivalent to a moderated ARDS. In a second group of studies sheep in which respiratory support was providing by a low flow-low pressure ECMO (ALung) partially rescued the animals returned the parameters of respiratory function to normal values. It is our goal to now use Alung in combination of MSCs to potentiate their protective effect.

15. SUBJECT TERMS
LPS induced ARDS, ALung, lung injury

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Introduction.

Transfer of injured service members from the Level 3 combat support hospital to level 4 and 5 medical facilities increase their chance of survival from devastating injuries. Aeromedical evacuation of patients with Acute Respiratory Distress Syndrome (ARDS) is sometimes beyond the possibilities because of limitations providing ventilator support in flight with a possible further deterioration in patient status. Cell based therapy with adult bone marrow-derived mesenchymal stromal cells (MSC) in experimental models of ARDS has been the focus of intense by investigation. Our previously published data suggest that administration of allogeneic MSCs can mitigate hypoxemia in ARDS and promote recovery. However, it is unknown how this new form of therapy can be used as an adjunct to current supportive measures for lung failure.

Our objective is to complete a series of preclinical studies in large animal models using two different protocols of extracorporeal membrane oxygenation (ECMO) alone or in combination with MSC in sheep. In a separate set of experiments, our collaborators in the USAISR in San Antonio, TX, will be using a pig model of ARDS in combination of a low flow ECMO. Our goal is to use a combination therapy of MSC and ECMO leading to a reduction in invasiveness of mechanical ventilation and inflammatory mediators as well as improvement in oxygenation and functional outcome.

Keywords

Acute respiratory distress syndrome (ARDS), extracorporeal membrane oxygenation (ECMO), Mesenchymal Stromal Cells (MSCs), transport injured service members.

Accomplishments

It is possible to summarize the achievements of each on of the four quarters to evaluate the progress of the project

First Quarter: Data collected until this quarter is from 22 preparations
Second Quarter: Seven new experiments for a total of 29.
Third Quarter: Six new experiments for a total of 33 experiments
Fourth Quarter: Eight new experiments for a total of 41 experiments

To our knowledge the data collected until this day is the first complete set of respiratory function in which the respiratory assisted devices, ECMO and ALung are compared on a large animal model of ARDS. We are including the data of blood oxygenation after endotoxin and mitochondrial function.

-First Quarter:

1. Coordination of the activities. Our group had multiple meetings with the different members to coordinate all the details of the experiment

2. We completed 7 preparations without complications. We believe the team is well trained and prepared to run each experiment.

-Second Quarter:

1. We completing the animals from each group. We proposed five groups each one with different conditions. Completion of each group is going to be considered as a Milestone.

-Third Quarter:

1. We had some issues with the cells, because the company delivered MSCs instead of MAPs, there is some concern if the efficacy of the cells is the same. We are planning a request to have a sixth group in which we test MSCs in LPS-induced ARDS.

For this **Fourth Quarter** these are our main achievements:

We had completed during the 2 years of the study a total of 41 preparations (**Table 1**). After a detailed analysis of the data of each experiment we had decided that 11 animals (experiments 2, 4 and 6) had to be excluded from the final analysis because they had complications, cardiac arrest and atrial fibrillations, during the preparation of the experiment before ARDS was induced. In some cases, the levels of blood oxygenation were below what, according to the approved protocol, is considered normal, a $P0_2$ below 300 mmHg is considered acute lung injury. Because these animals are coming from country farms, there is no absolute control on the health of the sheep. Animals that had a PO_2 below 300 mmHg did not receive LPS and were not included in the analysis. We consider these as complication were no related to the design of the experiment. Other animals had some level of complications, but those were not sufficient to exclude the data.

ID Sheep	Group	Excluded	Reason of exclusion
S16-01	LPS	Yes	Surgical + Low Pa/FiO2 Ratio before T0
S16-02	LPS	Yes	Low Pa/FiO2 ratio before T0 - NEVER GOT LPS
S16-03	SALINE		
S16-04	LPS	Yes	Arrhythmias - Low Pa/FiO2 ratio before T0
S16-05	LPS	Yes	Low Pa/FiO2 ratio before T0 - NEVER GOT LPS
S16-06	LPS		
S16-07	LPS		
S16-08	LPS		
S16-09	Alung	Yes	Low Pa/FiO2 ratio before T0 + Tracheal stenosis
S16-10	LPS		
S16-11	LPS+Alung		
S16-12	LPS+Alung		
S16-13	LPS+Alung		
S16-14	LPS+Alung		
S16-15	LPS + Alung	Yes	Problems in LPS delivery
S16-16	LPS+Alung		
S16-17	LPS+Alung		
S16-18	LPS+Alung		
S16-19	LPS+Alung		
S16-20	LPS		
S16-21	LPS		
S16-22	LPS		
S17-23	LPS		
S17-24	LPS		
S17-25	LPS + ECMO		
S17-26	LPS + ECMO		
S17-27	LPS+Alung		
S17-28	LPS+Alung		
S17-29	LPS + ECMO	Yes	Low Pa/FiO2 ratio before T0 - NEVER GOT LPS
S17-30	LPS + ECMO	Yes	Bronchoaspiration
S17-31	LPS + ECMO		
S17-32	LPS		
S17-33	LPS	Yes	Low Pa/FiO2 ratio before T0 - NEVER GOT LPS
S17-34	SALINE		
S17-35	LPS		
S17-36	LPS+Alung		
S17-37	LPS+Alung	Yes	Low Pa/FiO2 ratio before T0 - NEVER GOT LPS
S17-38	LPS+Alung	Yes	Ventricular Septal Defect detected upon Sac.
S17-39	LPS+Alung		
S17-40	LPS+Alung+MSC		
S17-41	LPS+Alung+MSC		

Since the experiment number 10 we had an anesthesiologist dedicated to the experiment, Tomas Drabek, who is an Associated Professor in the Department of Anesthesiology at the University of Pittsburgh joined our group. The incorporation of Dr Drabek had increased the reproducibility of the experiment. His main goal is to maintain the animal alive with the minimal intervention possible. This had allowed us to compare each one of the interventions (**Figure 1-3**).

Another important modification is that the group has regular meetings every 6-8 weeks to discus the project and analyzes the data. During the meeting the protocol is revised in detail to define if any modification needs to be implemented and to reaffirm the indications of when the intervention is going to be use. We review the data from each experiment for data analysis and quality control. In case that there is any level of uncertainty of a value, the clinical records are revised. During those meetings, we define implementation of small adjustments in the protocol, on sample collection and detailed review of the clinical records. This results and better coordination of the experimental team, consequently the quality of our data had improved.

On the ALung group two animals, numbers 9 and 15, did not received LPS. In the first case, to standardize the preparation, because this was a new protocol in which we were using for first time these type of cannulas, and to be sure that we were not inducing lung injury, at the moment of the surgery, we decided not to use endotoxin and cancel the experiment. By doing this we demonstrated that Alung along does not have any negative effect on a normal lung. On experiment 15 we did not observe any injury after LPS. After reviewing all the data of the experiment our conclusion was that the stock of the LPS used was thawed longer than was recommended, reducing the biological activity

Since experiment 11 we are measuring mitochondrial function on the lung and heart tissue (**Figure 4**). The Clark system is allowing us to measure the mitochondrial activity by the oxygen consumption during activation. Because the LPS-induced injury we observe a decline on mitochondrial function in specific compartments of the heart. Contrary to what was observed in the lung, where ALung was contributing to a small increase in the mitochondrial activity, suggesting a positive effect of ALung on mitochondrial function.

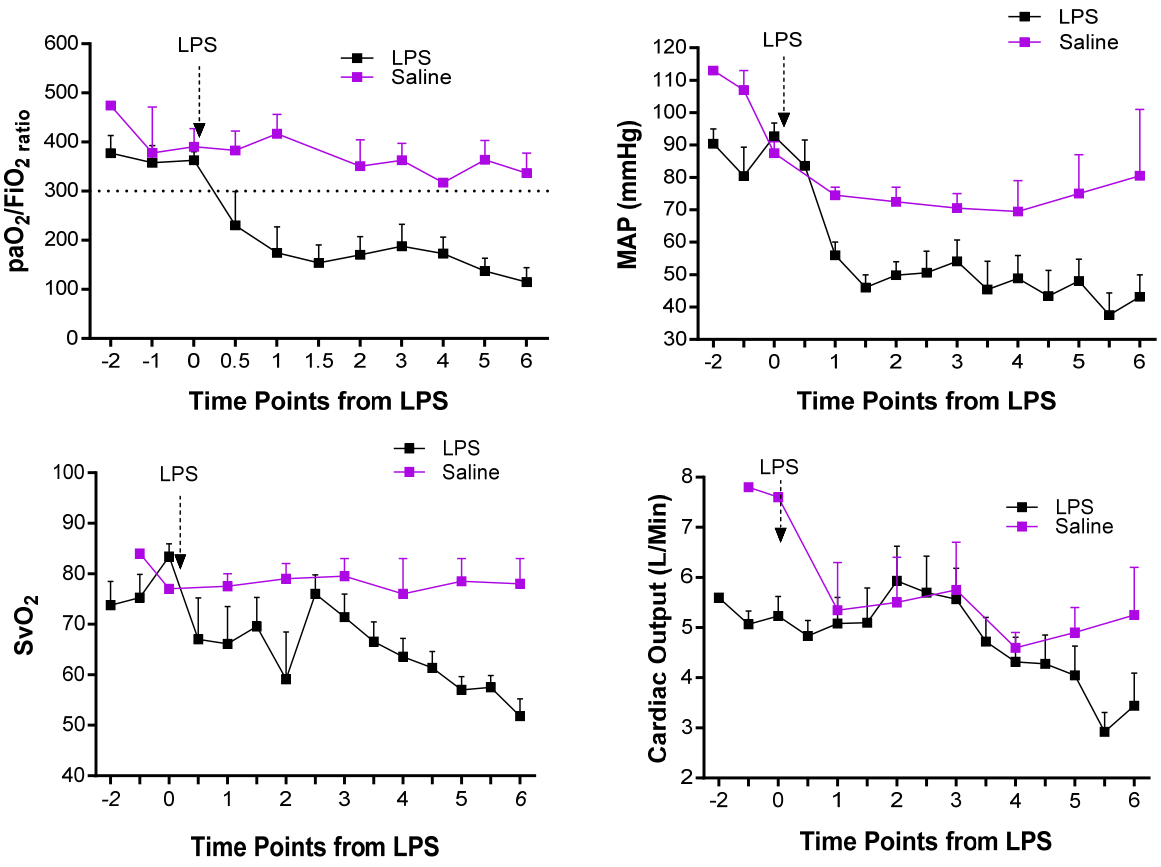


Figure 1. To define the level of injury induced by the new lot of LPS purchased for this project, we had to induce injury. We treated sheep with 3.5 ug/kg LPS systemically and followed the animal for six hours. LPS effect was compared with the values of animals that did note received LPS what were prepare using the same protocol. Blood oxygenation and levels of pCO2 decreases less than 30 min after infusion of LPS on all the parameters analyzed.

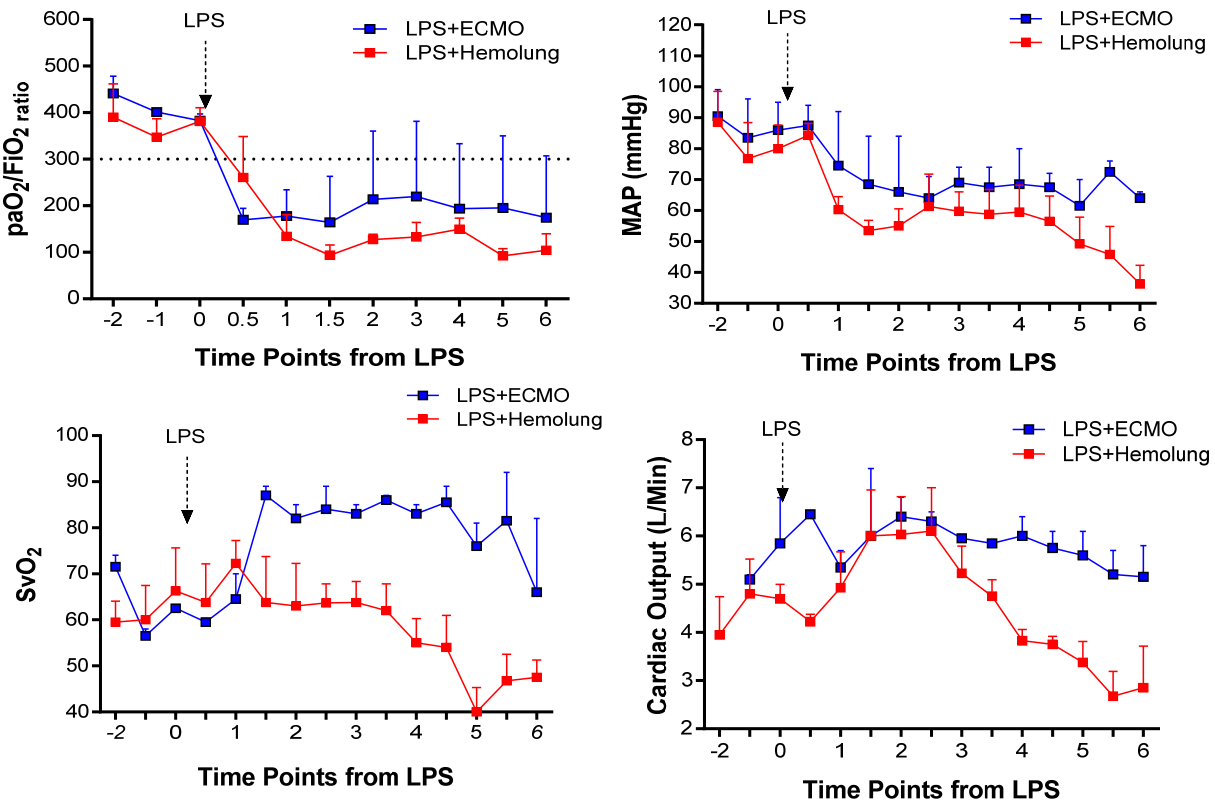


Figure 2. We are comparing the effect of the two pulmonary devices on animals on LPS-induced ARDS. There in minimal differences preserving respiratory function. Highest difference was observed in the cardioprotective activity of ECMO reflected in better SvO2 and cardiac output.

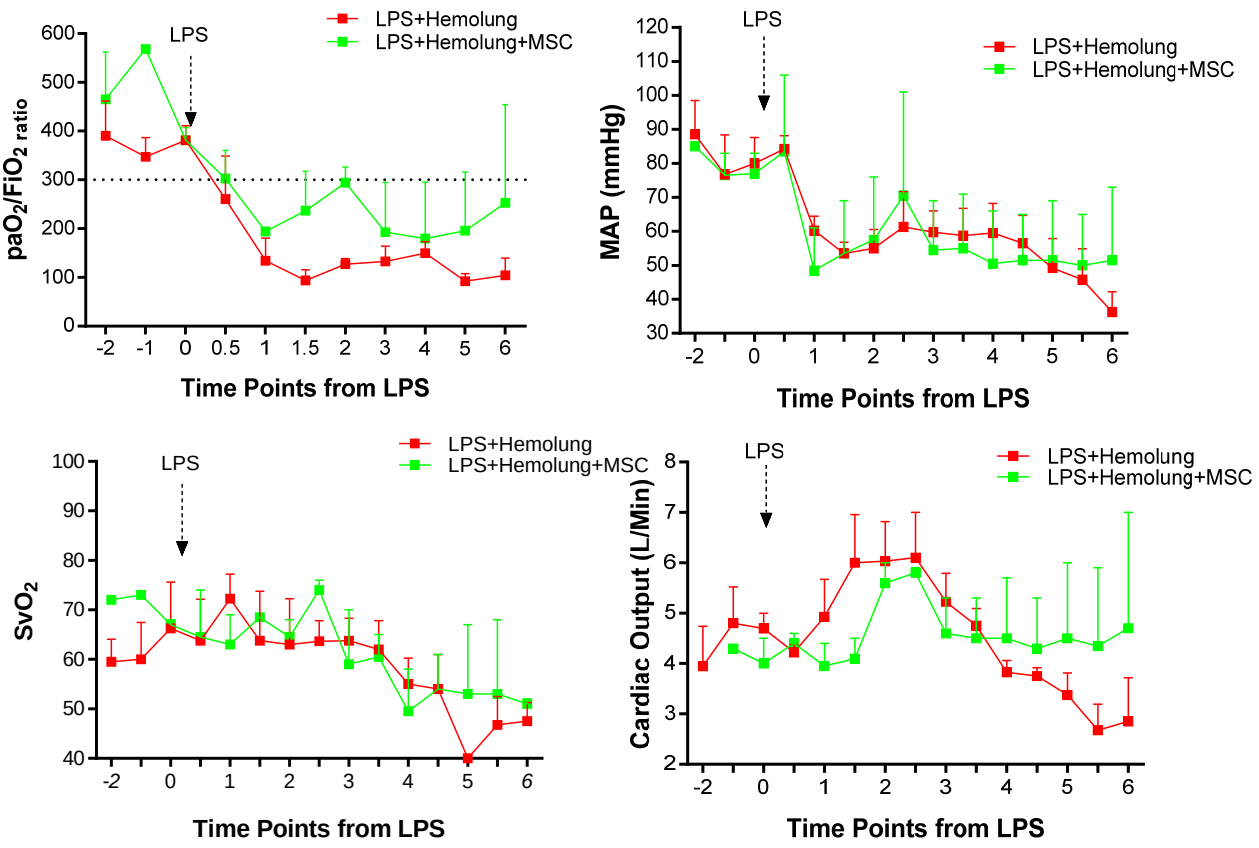


Figure 3. We had completed our first two experiments using MSCs in combination of Alung . Our preliminary data suggest an improvement in almost all the parameters analyzed when ALung was used in combination with MSCs compared to when ALung was used alone. These are still preliminary observations before final conclusions can be obtained from this group.

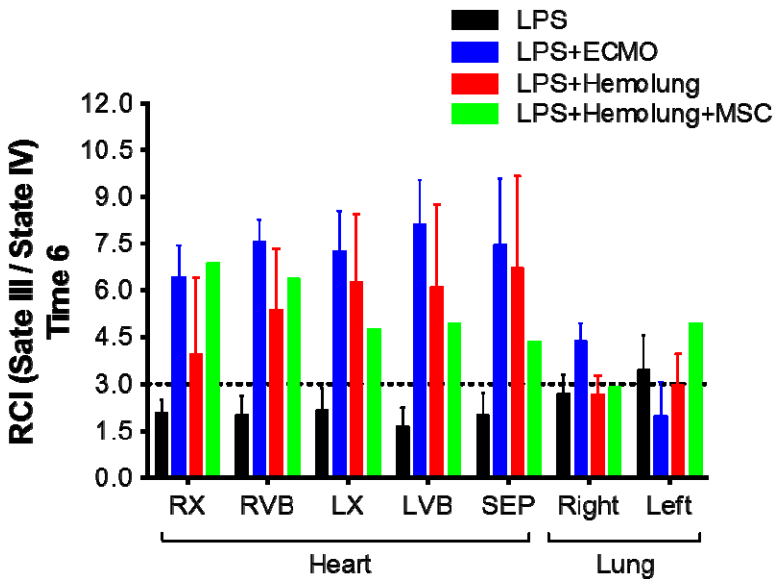
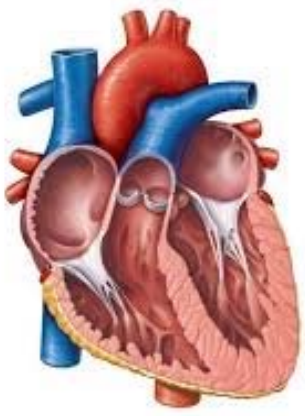


Figure 4. Mitochondrial activity is mostly preserved on the animals that were supported with ventilator devices and mesenchymal stem cells. Using a Clark system mitochondrial function was measured in different parts of the heart and each one of the lungs. RCI ration superior of 3 is associated with normal mitochondrial function, which was affected in all the samples on animals treated with LPS. All the interventions had a positive effect on the mitochondria. RX =Right ventricule apex, RVB=Right ventricule base, LX= Left ventricule apex, LVB= Left ventricule base, SEP=Septum.

Those are the initial analysis of the information collected in each experiment. The following in the list of all the parameters collected in each preparation.

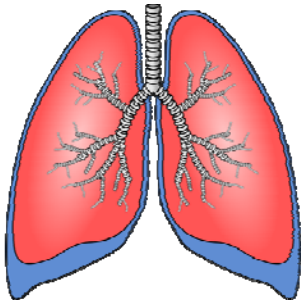


-Invasive continuous hemodynamic monitoring

- Blood Pressure (BP)
- Cardiac Output (CO)
- Mixed venous Saturation (SVO_2)
- Pulmonary Arterial Pressure (PAP)
- Central Venous Pressure (CVP)
- Body temperature
- Millar Pressure-Volume (PV) Loops system to measure ventricular pres

-Non-invasive hemodynamic monitoring

- Continuous Heart Rate (HR)
- Continuous Oxygen Saturation (SpO_2)
- Continuous EKG
- Fluids output and input
- Echocardiogram

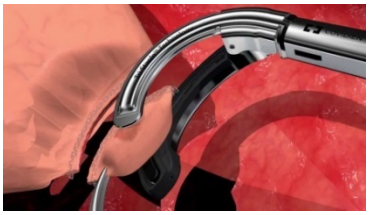


-Monitoring of Ventilation

- Tidal Volume
- Respiratory Rate
- Peak inspiratory Pressure
- Positive End Expiratory Pressure (PEEP)
- Fraction of inspired oxygen (FiO_2)

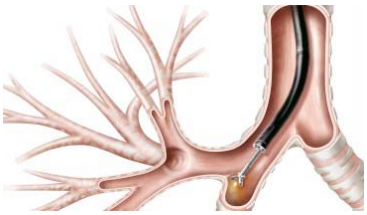
-Blood Samples

- Arterial Blood sample
  Arterial Blood gas status
- Mixed Venous Blood sample
  Blood gas status
  Plasma
  Differential cell count



-Lung and Heart Biopsy

- Wet to Dry
- Histology
- Protein and RNA
- Mitochondria function (Clark – Cyto – Mito – Complex assay)



-Fiber-optic bronchoscopy

- Airway Anatomy Evaluation
- Bronchoalveolar lavage (BAL)
  - Protein, RNA
  - Fluids quantification
  - Differential cell counts

Impact.

Development of new protocols to treat injured service member of the military forces can increase the survival and reduce long-term complications. In this initial phase of the study, we had confirmed that by using the proposed animal model we can evaluate the protective effect of any intervention.

As is presented in **Figures 1-3** we demonstrated that the model of injury of LPS-induced ARDS, proposed in this application, induces the changes in the respiratory and cardiac function consistent with ARDS. Except for the group LPS-ECMO-MSCs, we had realized preparations with all the protocols. As we expected pulmonary devices are not improving pulmonary function. In contrast, we observed an increase on cardiac function, what has not been reported before. This observation can have a large impact, because the impact of pulmonary rest is not resulting in a reduction on the time and severity of the lung injury. To overcome the lack of pulmonary protection by the pulmonary devices, in the next group of experiments we are including MSCs. In the initial group, we used ALung in combination of MSCs. Our preliminary data (n=2) suggest that MSCs are having the expected additive effect, that results in an improvement on pulmonary and cardiac function together.

In addition, this is the first study in which heart physiology is evaluated during ARDS; our data suggest that there is a decrease on mitochondrial function during ARDS which is reversed using any device and MSCs. Contrary, devices have a minimum effect on mitochondrial function on the lung (**Figure 3**).

Changes and Problems

As we described previously, some animals have intrinsic defects that results in cardiac arrhythmias which happened before injury was induced. Some animals were excluded of the analysis because were respiratory distress before the experiment was initiated, mostly because these climatic conditions in the farms that provide the animals.

Finally, because there is a small variation in the protocol used by the cell provider, we are planning to request a modification in the protocol in which we create additional group LPS-MSCs to demonstrate the level of protection by the cells alone. During the year using our own resources we purchased the equipment that was going to be requested.

Products

N/A

Participants & Other Collaborating Organizations

Personnel	Role	Percent Effort
Mauricio Rojas Associate Professor Department of Medicine McGowan Institute of Regenerative Medicine University of Pittsburgh	PI	38%
Jonathan D'Cunha Associate Professor of Cardiothoracic Surgery Vice Chair, Research and Education Chief, Division of Lung Transplant/Lung Failure Department of Cardiothoracic Surgery	Surgeon	25%
Ergin Kocyildirim Research Assistant Professor Department of Cardiothoracic Surgery	Surgeon	50%
Tomas Drabek Associate Professor Department of Anesthesiology	Anesthesiologist	5%
Bryan McVerry Assistant Professor of Medicine Associate Director Pulmonary and Critical Care Medicine Fellowship Program Director, Translational Research in Acute Lung Injury	Pulmonologist	5%
Nayra Cardenes Instructor Department of Medicine University of Pittsburgh	Coordinator	93%
Diana Alvarez Postdocotral Fellow	Postodoc	50%
Kentaro Nora Postdoctoral Fellow	Perfussionist	42%
Brian Kimball Technician	Technician	50%

Special Reporting Requirement

The system was already purchased with our own resources; this equipment is being used to determine number and viability of the MSCs after they are thawed and prepare for infusion. Measurements are being done in our facility providing reproducibility and precision required on this type cell based assays.

Appendices

N/A